JOURNAL OF BACTERIOLOGY, Jan. 1992, p. 415–425
0021-9193/92/020415-11$02.00/0

Vol. 174, No. 2

cysQ, a Gene Needed for Cysteine Synthesis in *Escherichia coli* K-12 Only during Aerobic Growth

ANDREW F. NEUWALD,[1,2] B. RAJENDRA KRISHNAN,[1] IGOR BRIKUN,[1] SAULIUS KULAKAUSKAS,[1]† KĘSTUTIS SUŽIEDĖLIS,[1]‡ TIHAMER TOMCSANYI,[1]§ THOMAS S. LEYH,[3] AND DOUGLAS E. BERG[1,4]*

Department of Molecular Microbiology, Box 8230,[1] Institute for Biomedical Computing,[2] and Department of Genetics,[4] Washington University Medical School, St. Louis, Missouri 63110-1093, and Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461[3]

Received 31 July 1991/Accepted 6 November 1991

The initial steps in assimilation of sulfate during cysteine biosynthesis entail sulfate uptake and sulfate activation by formation of adenosine 5′-phosphosulfate, conversion to 3′-phosphoadenosine 5′-phosphosulfate, and reduction to sulfite. Mutations in a previously uncharacterized *Escherichia coli* gene, *cysQ*, which resulted in a requirement for sulfite or cysteine, were obtained by in vivo insertion of transposons Tn*5tac1* and Tn*5supF* and by in vitro insertion of resistance gene cassettes. *cysQ* is at chromosomal position 95.7 min (kb 4517 to 4518) and is transcribed divergently from the adjacent *cpdB* gene. A Tn*5tac1* insertion just inside the 3′ end of *cysQ*, with its isopropyl-β-D-thiogalactopyranoside-inducible *tac* promoter pointed toward the *cysQ* promoter, resulted in auxotrophy only when isopropyl-β-D-thiogalactopyranoside was present; this conditional phenotype was ascribed to collision between converging RNA polymerases or interaction between complementary antisense and *cysQ* mRNAs. The auxotrophy caused by *cysQ* null mutations was leaky in some but not all *E. coli* strains and could be compensated by mutations in unlinked genes. *cysQ* mutants were prototrophic during anaerobic growth. Mutations in *cysQ* did not affect the rate of sulfate uptake or the activities of ATP sulfurylase and its protein activator, which together catalyze adenosine 5′-phosphosulfate synthesis. Some mutations that compensated for *cysQ* null alleles resulted in sulfate transport defects. *cysQ* is identical to a gene called *amtA*, which had been thought to be needed for ammonium transport. Computer analyses, detailed elsewhere, revealed significant amino acid sequence homology between *cysQ* and *suhB* of *E. coli* and the gene for mammalian inositol monophosphatase. Previous work had suggested that 3′-phosphoadenoside 5′-phosphosulfate is toxic if allowed to accumulate, and we propose that CysQ helps control the pool of 3′-phosphoadenoside 5′-phosphosulfate, or its use in sulfite synthesis.

The cysteine biosynthetic pathway (Fig. 1), a principal route of sulfur assimilation, involves more than 15 genes in at least five chromosomal regions in *Escherichia coli* and *Salmonella typhimurium*. It has been studied since the early days of physiological genetics in order to elucidate the roles of the individual genes, the control of their expression, and how the flow of metabolic intermediates is regulated (for a review, see reference 25). The transcription of most *cys* genes is positively controlled by the protein product of *cysB* and its coinducer, *O*-acetyl serine (also a cysteine precursor), during aerobic growth; transcription is repressed by sulfide, which is generated by reversal of the final biosynthetic step (Fig. 1). CysB seems not to be needed during anaerobic growth (3). The *cysQ* gene described here is also needed only during aerobic growth. It is inferred to act before sulfite formation, and hence this early part of the cysteine pathway is reviewed briefly below.

The initial step, sulfate uptake, is mediated by a permease encoded by the *cysT*, *cysW*, and *cysA* genes, which, along with *cysP*, constitute one operon (49). CysP protein is needed for maximal thiosulfate and sulfate binding, but it is probably not part of the permease, and its role in cysteine biosynthesis is unclear (19). A *cysZ* gene, about 10 kb from the *cysPTWA* operon, may also be needed for sulfate uptake (41). Intracellular sulfate is activated via synthesis of adenosine 5′-phosphosulfate (APS) by ATP sulfurylase, which is encoded by *cysD* and *cysN* (34). This activation step is complex, in that the rate of APS formation is greatly enhanced both by a protein activator (31, 32) and by GTP hydrolysis (33). APS is converted to 3′-phosphoadenosine 5′-phosphosulfate (PAPS) by APS kinase, encoded by *cysC*. This step is thought to not require cofactors, because APS kinase activity does not change during enzyme purification (46). Sulfite is generated from PAPS in a complex reaction involving transfer and reduction of its sulfuryl moiety. This reaction is catalyzed by the *cysH* gene product, PAPS sulfotransferase, and involves a thioredoxin- or glutaredoxin-bound intermediate (51, 52).

Strains with mutations in *cysH* or in both *trxA* and *grx* (encoding thioredoxin and glutaredoxin, respectively) grow poorly. The poor growth can be corrected by additional mutations in *cysC* (APS kinase) or genes for earlier steps in the pathway (16, 45a), a result indicating that PAPS or one of its derivatives is toxic if allowed to accumulate. We find this result interesting in the context of understanding mechanisms by which organisms cope with the many metabolic intermediates that are both essential for healthy growth and potentially deleterious. Other studies have shown that the activities of ATP sulfurylase and APS kinase decrease rapidly when growth is slowed (25, 26). Such instability could help modulate metabolite flow through this pathway and would be more sensitive to decreased need for PAPS

* Corresponding author.
† Permanent address: Institute of Applied Enzymology, FERMENTAS, Vilnius 232028, Lithuanian Republic.
‡ Permanent address: Department of Biochemistry and Biophysics, Vilnius University, Vilnius 232009, Lithuanian Republic.
§ Present address: Department of Zoology, Janus Pannonius University, Ifjusag uta 6, 7601 Pecs, Hungary.

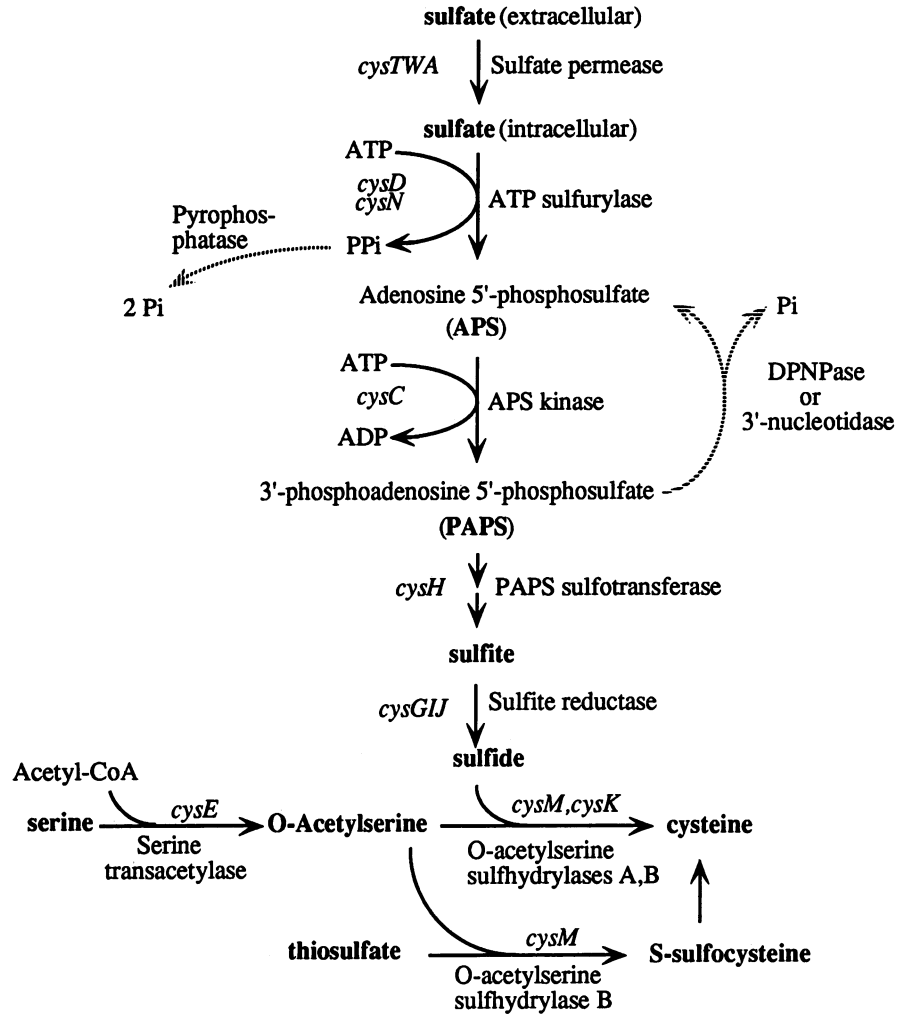

FIG. 1. Pathway of cysteine biosynthesis in *E. coli* (modified from data in references 25, 34, and 49).

than any transcriptional regulation. The dependence of APS synthesis (and thereby PAPS synthesis) on the ATP sulfurylase activator and on the local concentration of GTP (32, 33) might also help regulate PAPS levels.

Mutations in the *cysQ* gene described here result in a requirement for cysteine or sulfite that is expressed only during aerobic growth and that is leaky in many but not all laboratory strains of *E. coli*. Our studies suggest that CysQ may help control the levels of PAPS, its localization, or its use in sulfite synthesis.

MATERIALS AND METHODS

Strains, media, and general methods. The bacterial strains and plasmids used in this study are listed in Table 1. Bacteria were grown in LN broth (5) or Vogel-Bonner glucose-minimal salts medium (54). An M9-based minimal salts medium, with ammonium acetate in place of ammonium chloride (21), was used where indicated. Solid media contained 1.5% Difco Bacto-Agar. Antibiotics were used at the following concentrations: ampicillin, 250 μg/ml; kanamycin, 60 μg/ml; tetracycline, 12 μg/ml; streptomycin, 100 μg/ml; and chloramphenicol, 20 μg/ml. Isopropyl-β-D-thiogalactopyranoside (IPTG) was used at 0.5 mM. Amino acids were added at 50 μg/ml except for glycine, which was added at 200 μg/ml. Standard procedures were used for bacterial growth, characterization of auxotrophy and conjugation, DNA preparation, restriction endonuclease digestion, DNA electrophoresis, recombinant DNA cloning, and transformation (12, 47). All enzymes were obtained from commercial sources (Life Technologies, Inc., Stratagene, New England BioLabs, or Boehringer Mannheim) and used as directed. Anaerobic (H_2-CO_2 atmosphere) conditions were obtained by using BBL GasPak Anaerobic jars and the BBL GasPak plus system (Becton Dickinson and Co.).

Assays. The activity of 2′,3′-cyclic phosphodiesterase (encoded by the *cpdB* gene) was measured as the release of inorganic phosphate from cyclic UMP (4). Sulfate uptake was measured as depletion of $^{35}SO_4$ added to the medium with cells grown with djenkolic acid as the sulfur source and concentrated from the exponential phase (19). ATP sufurylase was measured as incorporation of $^{35}SO_4$ into PAPS, detected by thin-layer chromatography with dialyzed extracts of cells grown with sulfite as the sulfur source and induced with *O*-acetyl-1-serine (34). The level of the activator of ATP sulfurylase was also measured as PAPS synthesis

TABLE 1. Bacterial strains, phage, and plasmids

Strain, phage, or plasmid	Description or genotype	Source or reference
E. coli		
AJ2653[a]	ET8000 *amtA* (*cysQ*)::Tn*10*	21
BW6458	*proC*::Tn*5* *zje*::Tn*10*-BJW43 *metB1 relA1*	B. Wanner
CAG5052	Hfr *btuB*3191::Tn*10*, transfer counterclockwise from 7 min	48
DB747	W3350 *gal rpsL sup*^{0} (strain 594 of reference 7)	Laboratory collection
DB1434	DB747 (λ*plac5 cI857 S*am7)	28
DB4496	MC1061 *dam*::Tn*9* (p3)(pBRG1310)	43
DB5463	HfrH *lacZ*(Am) *trp*(Am) *sup*^{0}	D. Botstein (DB6128)
DB5508	*recD* (p3)	44
DB5659	DB747 *cysQ*::*kan*	λ*cysQ*::*kan* transduction
DB6302[a]	MG1655 *amtA* (*cysQ*)::Tn*10*	P1 transduction from AJ2653
DB6316	MG1655 *cysQ*::*kan*	λ*cysQ*::*kan* transduction
DB6908	ET8000 *cysQ*::*kan*	λ*cysQ*::*kan* transduction
DB6913	TG1 *cysQ*::*kan*	λ*cysQ*::*kan* transduction
DB6935	DB5508 *cysQ*::Tn*5supF*	This study
DB7101	TG1 Δ(*cysD-N*)::*kan*	λΔ(*cysD-N*)::*kan* transduction
DBan41	594 with *cysQ*::Tn*5tacI*	Tn*5tacI* transposition
DBan41TR	DBan41 with Δ (*srl-recA*)*306*::Tn*10*	P1 transduction from JC1289
DK21	*sup*^{0} *dnaB*(Am) *266* (λ*imm*^{21}-*ban*$_{P1}$)	29
ET8000	*rbs lacZ*::IS*1 gyrA hutC*$_k$	37
JC1289	Δ(*srl-recA*)*306* linked to Tn*10*	11
MC1061	F$^-$ *araD139*Δ(*ara-leu*)*7697* Δ*lacX74 galU galK hsdR hsdM rpsL*	8
MG1655	F$^-$ prototroph	17
TG1	F′ *proAB*$^+$ *traD*36 *lacI*^{q} *lacZ*ΔM15 *supE hsd*Δ5 *thi* Δ(*lac-proAB*)	47
BW6164	HfrRA2 *thr*::Tn*10*, clockwise transfer from 88 min	55
Phages		
M13mp18	Cloning vector	38
λ$^+$	λ wild type	Laboratory collection
λ::Tn*5tacI*	λTn*5tacI b*221 *c*I857 *O*am29 *P*am80	9
λ419	*cysA*$^+$ (5F7 of reference 24)	23
λ656	*cysQ*$^+$ (5B5 of reference 24)	23
λΔ(*cysD-N*)::*kan*	Derivative of λ652 (6C8 of reference 24)	28
λ*cysQ*::*kan*	Derivative of λ656	28
λ656*cysQ*::Tn*5supF*		This study
P1clr		Laboratory collection
Plasmids		
pBRGan101	Amp^r, *cysQ*::Tn*5tacI* (Kan^r) *cysQ*$^+$	pBR322 (*SalI* fragment from DBan41)
pBRGan102	Amp^r, *cysQ*::Tn*5tacI* (Kan^r) *cysQ*$^+$ *cpdB*$^+$	pBR322 (*ClaI* fragment from DBan41)
pBRGan103	Amp^r, *cysQ*$^+$ *cpdB*$^+$ *ClaI*::Tn*5tacI* Δ(0–52)	pBRGan102, small *ClaI* deletion (Kan^r)
pBRGan104	Amp^r, *cpdB*$^+$	pBRGan102, *EcoRI* deletion
pBRGan110	Amp^r, *cysQ*$^+$	pBR322 (*SalI* fragment of DB747)
pBRGan111	Amp^r, *cysQ*$^+$	pAN110, partial *MspI* digestion
pBRGan111-1 to pBRGan111-5	Amp^r, *cysQ*::*cat*	*cat* of pCM4 ligate into partial *Sau*3A of pBRGan111 (Fig. 2 and 4)
p*cysQ*::*kan*	Amp^r, *cysQ*::*kan*	*kan* of pUC4K into *Eco*RI site of pBRGan111
pCM4		10
pBR322	Amp^r Tet^r	6
p3	Kan^r *amp*(Am) *tet*(Am)	29
pBRG1310	Tn*5supF* donor	43

[a] The *amtA* gene is identical to *cysQ*, as detailed in the text.

in reactions containing purified ATP sulfurylase and dialyzed cell extracts as the source of ATP sulfurylase activator (31, 32).

Genetic manipulation and analysis. Standard methods were used for (i) mutagenesis of *E. coli* with Tn*5tacI* with phage λ::Tn*5tacI* b221 *c*I857 *O*am29 *P*am80 as a transposon donor (9) and (ii) Hfr conjugation and P1 generalized transduction (48). Cysteine-requiring bacteria were tested for sensitivity to azaserine and to chromate by spotting dilutions of these agents on lawns of 10^7 bacteria spread on minimal glucose agar supplemented with cysteine, djenkolic acid, or glutathione and IPTG, as appropriate, and by growth in liquid cultures with progressive twofold differences in the concentrations of these agents.

To insert a transcription reporter into the *cysQ* gene, the DNA of *cysQ* plasmid pBRGan111 was partially digested with *Sau*3A, and full-length linear DNA was isolated after electrophoresis in low-melting-point agarose and ligated to the *Bam*HI *cat* fragment from plasmid pCM-4 (10). The

religated DNA was used to transform the *cysQ*::Tn*5tac1* strain DBan41, and plasmids that did not complement its cysteine auxotrophy were identified and characterized.

To generate a *cysQ*::Tn*5supF* insertion mutant, Tn*5supF* was transposed from the donor plasmid in strain DB4496 (43) to *cysQ*$^+$ phage λ656 (23, 24), and insertion-containing phage were selected by plaque formation on the *dnaB* amber strain DK21 (29, 43). Haploid Tn*5supF*-containing bacterial recombinants were obtained by infecting strain DB5508 (which contains amber mutant alleles of *amp* and *tet* genes) and selecting Sup$^+$ transductants by their resistance to ampicillin or tetracycline (44). Sup$^+$ transductants were screened for auxotrophy. To generate a *cysQ*::*kan* mutant, an *Eco*RI *kan* cassette from plasmid pUC4K was ligated into the *Eco*RI site in *cysQ* of pBRGan111. This allele was recombined into λ656 by infecting cells carrying the pBRGan111-*cysQ*::*kan* plasmid and selecting phage carrying the *cysQ*::*kan* allele by transduction of DB1434. λ*cysQ*::*kan* phage recovered from the lysogen were used to transduce nonlysogens and thereby obtain haploid *cysQ*::*kan* bacteria (28).

Cys$^+$ revertants of *cysQ* mutant strains were obtained by growing young single-colony isolates in 2 ml of LN broth to stationary phase, washing the cells twice with 10 mM $MgSO_4$, plating aliquots on minimal (cysteine-free) medium, and incubating for 2 days at 37°C. Reversion frequencies were measured by using several cultures from different single colonies to avoid jackpots.

DNA sequence analysis. A 1-kb segment containing the *cysQ* gene was sequenced by the Sanger dideoxynucleotide-chain termination method with Sequenase (U.S. Biochemical, Cleveland, Ohio) and single- and double-stranded DNA templates (27). Primer binding sites were provided by insertions of transposons Tn*5tac1* and Tn*5supF* in phage λ656, by the promoterless *cat* gene in plasmid pBRGan111 DNAs, and by a universal primer binding site in M13mp18 (38) (for sequencing an *Eco*RI-*Pst*I fragment containing the 3′ end of *cysQ*).

The oligonucleotides used as sequencing primers are as follows: (i) 5′ CTCCATTTTAGCTTCCTTAGCTCC, positions 40 through 17 at the 5′ end of the *cat* gene cassette; (ii) 5′ TGTCAAAACATGAGAATTCCTCCCG, positions 43 through 20 near the I end of Tn*5tac1*; (iii) 5′ GGAAACAGA ATTCCCGGGGATCCCC, positions 4549 through 4573 near the O end of Tn*5tac1*; (iv) 5′ TAGGATCCCCTACTTGT GTA, positions 30 through 11 near the O end of Tn*5supF*; (v) 5′ TAGGATCCCGAGATCTGATC, positions 236 through 255 near the I end of Tn*5supF*; (vi) 5′ GAGCGGCC AAAGGGAGCAGAC, positions 139 through 159 (middle primer) within Tn*5supF* with its 3′ end toward the I end; (vii) 5′ GTAAAACGACGGCCAGT, the universal M13 sequencing primer.

Nucleotide sequence accession number. The nucleotide sequence of the *cysQ* gene shown in Fig. 4 has been deposited with GenBank under accession number M80795.

RESULTS

Initial detection and characterization of *cysQ*. The prototrophic strain *E. coli* DB747 was mutagenized with Tn*5tac1*, a transposon with an outward-facing *tac* promoter that is regulated by the *lac* repressor and IPTG (9). A conditional mutant that required cysteine for growth on minimal medium containing IPTG, but not on medium lacking IPTG, was isolated and named DBan41. Early characterizations of this strain revealed two other novel features. It did not require cysteine for normal growth in an anaerobic atmosphere. In addition, it formed slow-growing colonies on cysteine-free medium containing IPTG (after 2 to 3 days, instead of 16 h in the case of its Cys$^+$ parent). Cells in these colonies exhibited the same slow-growth phenotype, which indicated that the mutation was leaky, not highly revertible. The addition of IPTG to DBan41 in cysteine-free liquid medium lengthened the cell doubling time from about 80 min to 280 min.

The cysteine requirement of DBan41 was satisfied by sulfite at 0.3 mM, which indicated a defect in the sulfate assimilation branch of the pathway (Fig. 1). The strain was as sensitive to chromate (MIC, 50 to 100 μM) as its wild-type parent and also grew on low concentrations of thiosulfate (1 to 2 mM). Sulfate uptake mutants are chromate resistant, and many are deficient in thiosulfate uptake (14, 40); therefore this mutation seemed to affect a step leading to sulfite that follows sulfate uptake (Fig. 1).

The mutation was mapped by genetic and molecular methods. (i) The *cys*$^+$ allele was transferred efficiently by Hfr strains BW6164 and CAG5052 to DBan41, which placed the mutation in the 88- to 07-min interval of the *E. coli* chromosome, far from other known cysteine biosynthetic genes (1). (ii) The *cys*$^+$ allele was cotransduced by phage P1 at a frequency of 1% with *zje*::Tn*10*, an insertion at 94 to 95 min (strain BW6458). (iii) The *cys*$^+$ allele was also efficiently transduced by λ656 (23, 24), a λ phage clone that carries the segment of the *E. coli* chromosome from kb ~4511 to kb ~4525 (near 96 min). The mutant allele was recessive to the wild type in partial diploids, which were formed by λ656 infection of a λ$^+$ lysogenic derivative of DBan41 (44), as well as in strains carrying the wild-type allele in multicopy plasmids.

More refined map information came from molecular cloning (Fig. 2). (i) Kan^r plasmids obtained by cloning *Sal*I- or *Cla*I-digested DBan41 DNA in pBR322 contained an 18-kb *Sal*I fragment (pBRGan101) or an overlapping 14-kb *Cla*I fragment (pBRGan102), respectively. (ii) A plasmid obtained by cloning *Sal*I-digested wild-type *E. coli* DNA (pBRGan110) that complemented the *cysQ*::Tn*5tac1* allele contained a 14-kb fragment whose restriction map matched that of the chromosome adjacent to the Tn*5tac1* insertion. (iii) A deletion plasmid that retained only 1.8 kb of chromosomal sequence but retained Cys$^+$ complementing activity was generated by partial *Msp*I digestion of pBRGan110 DNA (pBRGan111). Comparisons of the restriction digest patterns of these clones with the known restriction map of the chromosomal region near 96 min (24, 36) indicated that Tn*5tac1* was at kb 4517, about 900 bp upstream of the *cpdB* gene. Transcription from the *tac* promoter in Tn*5tac1* was toward *cpdB* (clockwise).

Cys$^-$ phenotype not caused by *cpdB* overexpression. The CpdB protein has a 3′-nucleotidase activity that can degrade PAPS to APS in vitro (4). Although CpdB protein seems to be primarily periplasmic, findings of cytoplasmic inhibitors for other periplasmic nucleotidases (36) suggested models in which CpdB also acted intracellularly. Thus, in principle, transcription from the *tac* promoter might cause a cysteine requirement by increasing *cpdB* expression. Alternatively, it might alter the expression of an unknown gene next to *cpdB*. Three findings eliminated the simple CpdB-based model of cysteine auxotrophy: (i) the CpdB level in strain DBan41 was increased less than 2-fold by IPTG (data not shown); (ii) the multicopy *cpdB*$^+$ plasmid (pBRGan104) did not cause a cysteine requirement, although it did result in 10-fold higher CpdB activity (data not shown); and (iii) the cysteine requirement was complemented by plasmid pBRGan102,

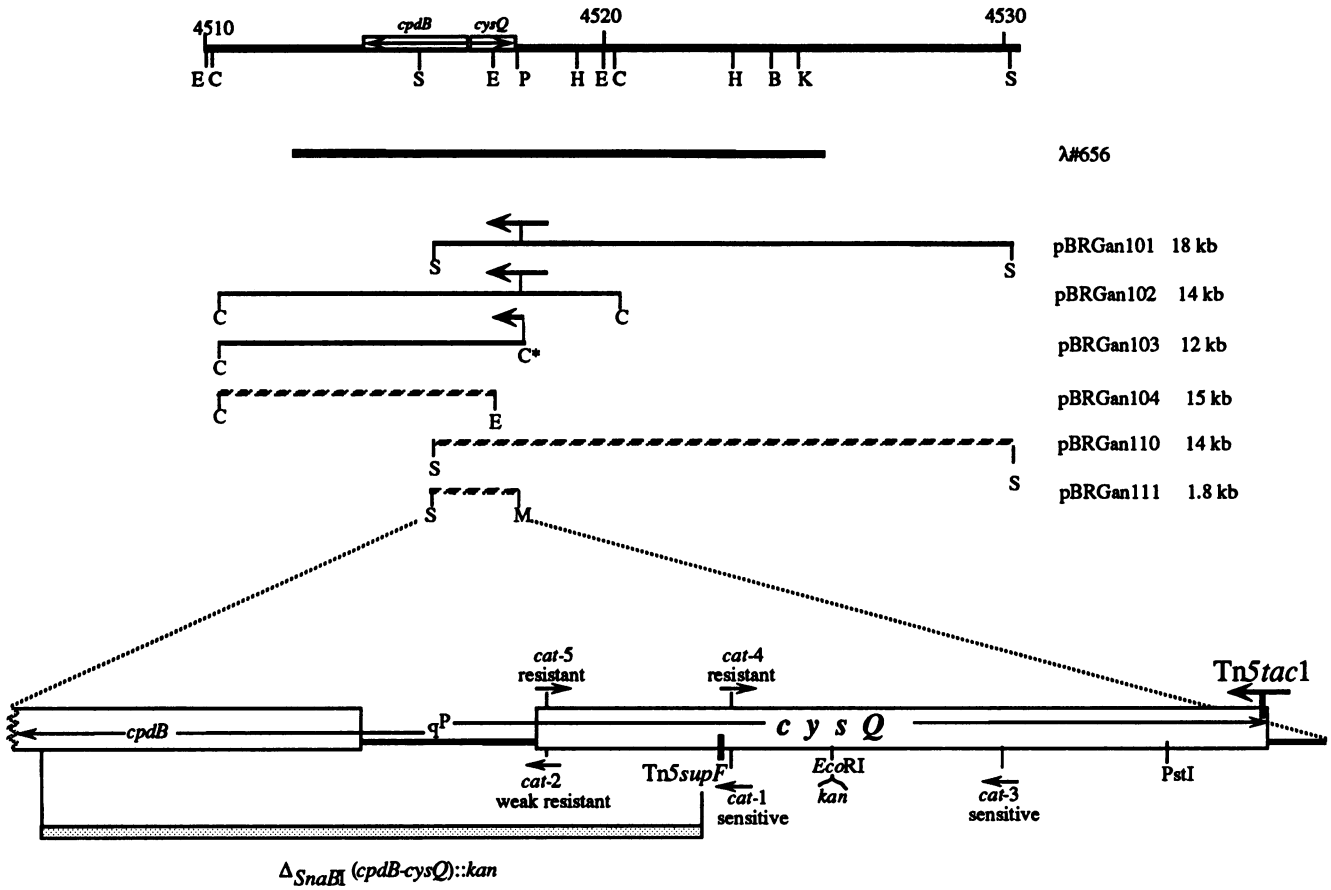

FIG. 2. Physical map of the *cysQ* region of *E. coli*. The extents of bacterial DNA cloned in the phage λ656 and in the plasmids used in this work are depicted, as are the positions of insertions and deletions that helped define *cysQ*. E, *Eco*RI; C, *Cla*I; S, *Sal*I; H, *Hin*dIII, B, *Bam*HI; K, *Kpn*I. The arrows indicate directions of transcription: heavy arrow, from the *tac* promoter in Tn*5tac1*; long arrows, *cysQ* and *cpdB*; smaller arrows, orientations of transcription of a *cat* reporter inserted at *Sau*3A sites in *cysQ*.

which contains a chromosomal segment including *cpdB* and the Tn*5tac1* insertion. These results implied that the cysteine requirement was due to altered expression of a previously unknown gene next to *cpdB*. This gene was designated *cysQ*.

Direction of *cysQ* transcription. Insertions of a *cat* reporter gene were made to determine the orientation of *cysQ* and thereby to deduce whether auxotrophy resulted from overexpression or underexpression of *cysQ* after IPTG-induced transcription from the *tac* promoter in mutant strain DBan41. Five different insertions into *Sau*3A sites of plasmid pBRGan111 that inactivated Cys^+ complementation activity were isolated; restriction mapping showed that each was within about 600 bp of the start of *cpdB* (Fig. 2). Insertions 4 and 5, oriented away from *cpdB* (toward Tn*5tac1*), conferred chloramphenicol resistance (25 μg/ml), whereas insertions 1 and 3, in the opposite orientation, did not. Insertion 2, also in the opposite orientation but closest to *cpdB*, conferred weak resistance (~10 μg/ml). This was attributed to a second promoter in *cysQ* that could allow *cpdB* transcription (see sequence analysis, below). Based on insertions 1, 3, 4, and 5, we inferred that *cysQ* is transcribed toward Tn*5tac1*.

Phenotypes conferred by *cysQ* null alleles. Chromosomal *cysQ* null mutations were generated and used to assess whether the distinctive leaky auxotrophy and its correction by anaerobic growth were allele or gene specific. (i) A *cysQ*::Tn*5supF* insertion allele was obtained by selecting transposition of Tn*5supF* to the $cysQ^+$ phage λ656 (29, 43). One of 50 Tn*5supF* insertions resulted in cysteine auxotrophy when recombined into the *E. coli* chromosome, and DNA sequencing (see below) showed that Tn*5supF* was inserted in *cysQ*. (ii) A *cysQ* null allele marked with kanamycin resistance was made by insertion of a *kan* gene at the *Eco*RI site in pBRGan111 (Fig. 2), recombined from the plasmid into λ656, and then recombined from the *cysQ*::*kan* phage into bacterial chromosomes (28). (iii) A segment containing part of both the *cpdB* and the *cysQ* genes was deleted (Δ*Sna*BI; Fig. 2) to further test a possible involvement of *cpdB* in the *cysQ* mutant phenotype. This deletion was marked by insertion of *kan* and recombined into λ656 and from there into bacterial chromosomes. Finally, E. Barnes kindly provided us with a fourth *cysQ* null allele, which was generated by Tn*10* (Tet^r) insertion and was originally designated *amtA*::Tn*10* (21, 22).

Each of these four *cysQ* null alleles resulted in a cysteine requirement that was leaky, corrected by anaerobiosis, and satisfied by sulfite when transduced into several *E. coli* K-12 strain backgrounds, including DB747 (used to isolate the original Tn*5tac1* insertion), DB5508, and MG1655. These alleles were much less leaky in two other laboratory strains: TG1 and ET8000. The strain background determines the leakiness of the *cysQ* mutant phenotype; *cysQ* derivatives of

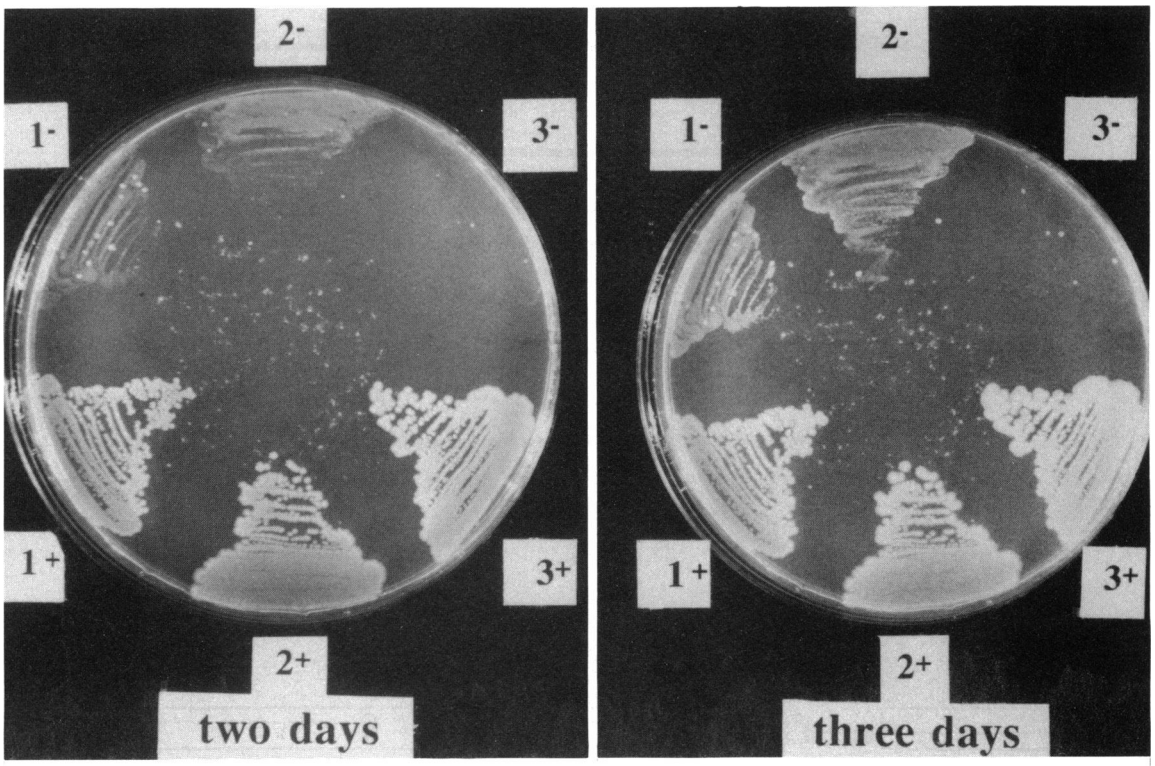

FIG. 3. Growth of *cysQ*::*kan* mutants and wild-type parents. Growth was for 2 or 3 days at 37°C on M9 salts glucose medium (no added cysteine or sulfite). (Scattered white dots near the center of plate are a salt precipitate that often form in M9 solid medium.) The numbers 1, 2, and 3 indicate strain backgrounds DB747, MG1655 and ET8000, respectively. −, *cysQ*::*kan*; +, Cys^+.

DB747 and MG1655 showed some growth on cysteine-free medium after 2 days at 37°C and small single colonies after 3 days, whereas the corresponding derivative of ET8000 showed barely perceptible growth only after 3 days (Fig. 3). All *cysQ* mutant strains grew normally on sulfite or under anaerobic conditions. The Cys^- phenotype caused by the Δ(*cysQ cpdB*) allele was identical to that caused by simple insertions in *cysQ* in these strain backgrounds.

The match between the null mutant phenotype and that of the Tn*5tac1* insertion indicated that IPTG-induced transcription from Tn*5tac1* shuts off the expression of *cysQ* quite completely. The equivalence of Cys^- phenotypes of the Δ(*cysQ cpdB*) and the simple *cysQ*::*kan* insertion alleles ruled out a model in which CpdB protein would consume PAPS and in which CysQ protein would regulate this consumption.

DNA sequence of *cysQ*. A 1-kb segment containing the sites of insertion mutations that defined *cysQ* was sequenced by using primer binding sites provided by the Tn*5tac1* and Tn*5supF* transposons, the *kan* and *cat* insertions, and, for one segment, an M13mp18 vector. All portions of this segment were sequenced on both DNA strands. The *cysQ* DNA sequence corresponded to a 246-codon open reading frame preceded by sequences that match consensus transcription promoters and translation initiation sites (Fig. 4). The first part of this sequence matched that found earlier in the 0.5 kb upstream of *cpdB* in *E. coli*; analyses of *S. typhimurium* indicated a *cysQ* homolog in the same location in this species (35). The *cysQ* sequence we determined was identical to that reported for *amtA* (15).

The DNA sequence confirmed that transcription of *cysQ* should diverge from that of *cpdB*, as suggested by *cat* reporter insertions. Tn*5tac1* was inserted within *cysQ*, just two codons from its 3′ end (a fusion protein with 17 additional amino acids is predicted). Tn*5supF* was inserted at codon 72 of *cysQ*. Only 17 bp separate the −35 regions of putative promoters for *cysQ* and *cpdB*, and an apparent consensus cyclic AMP receptor protein (CRP) binding site (placed such that CRP binding could stimulate *cpdB* transcription; see Fig. 4 legend) (35) overlaps the putative *cysQ* promoter. The low-level chloramphenicol resistance associated with the *cat* insertion 2 is attributable to an additional promoter within *cysQ* (nucleotides 72 to 46, underlined in Fig. 4). (This promoter might also explain the relative weakness of the CRP-cyclic AMP dependence of *cpdB* expression [35].) Binding sites for the CysB positive regulatory protein, found in the promoter regions of most cysteine biosynthetic genes (18), did not seem to be present in the *cysQ* promoter region. A search of the PROSITE data base of protein motifs (2) did not reveal significant matches to CysQ. However, we recently found strong amino acid sequence level homologies between *cysQ* and *suhB* of *E. coli* (Fig. 5), mutations in which suppress certain *rpoH* missense alleles (apparently by elevating the levels of heat shock sigma subunit of RNA polymerase that it encodes [56]) and also between *cysQ* and genes for several eukaryotic proteins, including inositol monophosphatase (13, 39).

Does *cysQ* participate in ammonium uptake? While preparing this manuscript, we learned that *cysQ* corresponds to the gene called *amtA* (21, 22), a designation based on the finding of a Tn*10* insertion mutation (*amtA*::Tn*10*) that blocked growth on minimal (cysteine-free) medium containing very low levels of ammonium (≤0.1 mM, rather than the ≥10 mM used in most media). It was proposed that *amtA* is needed

FIG. 4. DNA sequence of the *cysQ* gene. Sites of insertion and orientations (in cases of transposons) are indicated, as are putative promoters for *cysQ* and *cpdB* and the consensus CRP binding site. The CRP binding site identified in ref. 35 extends from positions −72 to −96, relative to the start of *cysQ* translation (beginning at TT in the −35 region of the *cysQ* promoter). The DNA sequencing protocols and sequences of the oligonucleotide primers used are given in Materials and Methods.

for active ammonium uptake (21). Our reconstruction experiments showed, however, that even *cysQ*$^+$ (*amtA*$^+$) bacteria grew very poorly on low-ammonium medium (Fig. 6). Strains with the *amtA*::Tn*10* or *cysQ*::*kan* insertion mutations failed to form colonies on this medium, as reported earlier (21). The mutants did grow, however, when this medium was supplemented with cysteine (Fig. 6) or sulfite, in which case these mutant strains were indistinguishable from their Cys$^+$ parents in colony size. Since neither sulfite nor cysteine can be used as an ammonium source by *E. coli* (53), these results are not consistent with the interpretation (21) that the AmtA (CysQ) protein is needed for ammonium uptake.

Possible roles for *cysQ*. We tested whether *cysQ* might be needed in sulfate uptake or activation. No effect of *cysQ* null mutations was found on the rapid uptake of labelled sulfate from the medium in the background of strain TG1 or DB5463 (nonleaky and leaky cysteine requirements, respectively). In contrast, a *cysDN* (ATP sulfurylase) deletion strain was severely deficient in sulfate uptake, as expected (14) (data not shown). No significant differences between *cysQ* mutant and parental strains were detected in levels of ATP sulfurylase, which catalyzes synthesis of APS, the first activated sulfur intermediate. *cysQ* mutations also had no effect on the level of the activator of ATP sulfurylase (data not shown).

Reversion of *cysQ* null mutants. Cys$^+$ revertants of

```
   MLDQVCQLARNAGDAIMQVYDGTKPMDVVSKADNSPVTAADIAAHTVIMDG     (1-51)    CysQ
   ML      AR AG  I   Y+    ++   K+ N  VT  D AA  VI+D
MHPMLNIAVRAARKAGNLIAKNYETPDAVEASQKGSNDFVTNVDKAAEAVIIDT    (1-54)    SuhB

LRTLTPDVPVLSEEDPPGWEVRQHWQRYWLVDPLDGTKEFIKRNGEFTVNIALI    (52-105)  CysQ
+R   P   +++EE     E         W++DPLDGT  FIKR   F V IA+
IRKSYPQHTIITEE-SGELEGTDQ-DVQWVIDPLDGTTNFIKRLPHFAVSIAVR    (55-106)  SuhB

DHGKPILGVVYAPVMNVMYSAAEGKAWKEECGVRK-QIQVRDARPPLVVISRSH    (106-157) CysQ
 +G+  ++VVY P+ N +++A  G +     G R      RD    ++
IKGRTEVAVVYDPMRNELFTATRGQG-AQLNGYRLLGSTARDLDGTILATGFPF    (107-159) SuhB

ADAEL KEYLQQLGE-----HQTTSIGSS-LKFCLVAEGQAHVYPRFGPTNIWD    (158-205) CysQ
       Y++ +G              GS  L    VA G    +   G    WD
KAKQYATTYINIVGKLFNECADFRRTGSAALDLAYVAAGRVDGFFEIG-LRPWD    (160-212) SuhB

TAAGHAVAAAAGAHVHDWQGKPLDYTPRESFLNPGFRVSIY                 (206-246) CysQ
 AAG  +   AG+ V D  G    Y      +    RV
FAAGELLVREAGGIVSDFTGGH-NYMLTGNIVAGNPRVVKAMLANMRDELSDALKR  (213-267) SuhB
```

FIG. 5. Alignment of amino acid sequences of inferred protein products of *cysQ* and *suhB* (adapted from data in reference 39). Identities are indicated by placements of conserved amino acids in the middle line; conservative substitutions (+) are indicated. Overlined segments indicate regions with high sequence similarity to inositol monophosphatase (13). The CysQ (SuhB) initial amino acid alignment score of 229, calculated by using the FASTA program (42), was 28 standard deviation units above the mean initial score of 24.6 for comparisons of CysQ to the other sequences in the PIR (release 28) protein data base. In a test using the Dayhoff Relate program, there were four segments of 25 amino acids in length that were more than eight standard deviations above the mean. This indicates strong homology: the probability of getting a single segment with such a deviation from a random sequence by chance alone is less than 10^{-15} (42).

cysQ::*kan* and *cysQ*::Tn*10* mutants were obtained at frequencies of about 10^{-6} with derivatives of TG1 and ET8000 (nonleaky *cysQ* mutant phenotype) and $>10^{-5}$ with derivatives of DB747 and MG1655 (leaky *cysQ* mutant phenotype); these differences in recovery probably reflect the greater leakiness of *cysQ* mutations in the DB747 and MG1655 backgrounds. The revertants were heterogeneous in colony size on cysteine-free medium but grew as well as their $cysQ^+$ ancestors on cysteine-containing medium and retained the Kan^r or Tet^r traits of their Cys^- parents. The parental *cysQ* mutant alleles were recovered from several revertants by transduction and selection for the appropriate resistance trait (Kan^r or Tet^r). Two spontaneous reversion mutations that allowed relatively good growth on cysteine-free medium were mapped in Hfr × F^- crosses and then by transduction with several candidate λ phage clones (marked by insertion of a Tn*5cam* transposon [44, 50]). These reversion mutations were found to be in the segment carried by λ419, a phage clone that also carries the *cysPTWA* (sulfate binding and uptake) operon. The two revertants tested were found to be defective in sulfate uptake, unlike their cysteine-requiring parents. Partial diploids generated by lysogenizing revertants with λ419::Tn*5cam* and a λ^+ helper required cysteine, indicating that the reversion mutation is recessive and thus probably due to loss of function. This Cys^- phenotype was unstable, however, because of frequent homogenotization for the parental (nonrevertant) allele.

DISCUSSION

The initial steps in the sulfate assimilation branch of the cysteine pathway entail sulfate uptake, its activation via formation of APS and conversion to PAPS, and then its reduction to sulfite (Fig. 1). The mutational and sequence analyses presented here identified a previously uncharacterized gene, *cysQ*, whose product is needed for proper metabolic functioning of this part of the pathway. We propose below that CysQ acts on PAPS. The *cysQ* gene was mapped to a locus at ~96 min in the *E. coli* chromosome, which is far from other *cys* genes. The *cysQ* promoter region overlapped a CAP binding site that is implicated in the control of expression of the adjacent gene, *cpdB* (35), and it did not contain a good match to the consensus binding site for the CysB regulatory protein (18). Hence, the expression of *cysQ* may be controlled differently from that of most other *cys* genes. The auxotrophy resulting from mutations in *cysQ* was leaky in some strain backgrounds and was compensated by mutations in other genes; *cysQ* mutants were prototrophic during anaerobic growth.

A precedent for cysteine biosynthetic genes that are not needed during anaerobic growth is provided by *cysI* and *cysJ* in *S. typhimurium* (3). This case reflects the presence of additional anaerobic sulfite reduction (*asr*) genes. *E. coli* lacks such *asr* genes (20), however, and our studies indicate that *cysQ* acts before, not after, sulfite formation (Fig. 1). *cysB* is also only needed during aerobic growth (3), suggesting that a separate transcriptional activator may be operating anaerobically. *cysQ* does not seem to be a transcriptional activator of *cys* genes, since it does not significantly affect the rate of sulfate uptake or the level of ATP sulfurylase or its protein activator.

cysQ is identical to *amtA*, a gene which had been thought to participate in ammonium transport (21). That interpretation was based on a failure of mutant strains to grow on low-ammonium cysteine-free medium or to take up methylammonium at a high rate when they were grown with arginine in place of ammonium (21). We found that the growth defect of *cysQ* (*amtA*) mutants was compensated by sulfite or cysteine (Fig. 6), neither of which serves as a nitrogen source (53). The initial failure to recognize the cysteine requirement of the *amtA* mutant (21) may have been due to leakiness on normal minimal medium (Fig. 3) or inadvertent selection of a (partially) compensating suppressor mutation. The inability of *cysQ* (*amtA*) mutants to grow on low-ammonium medium probably results from the combined effects of partial starvation for ammonium (because of

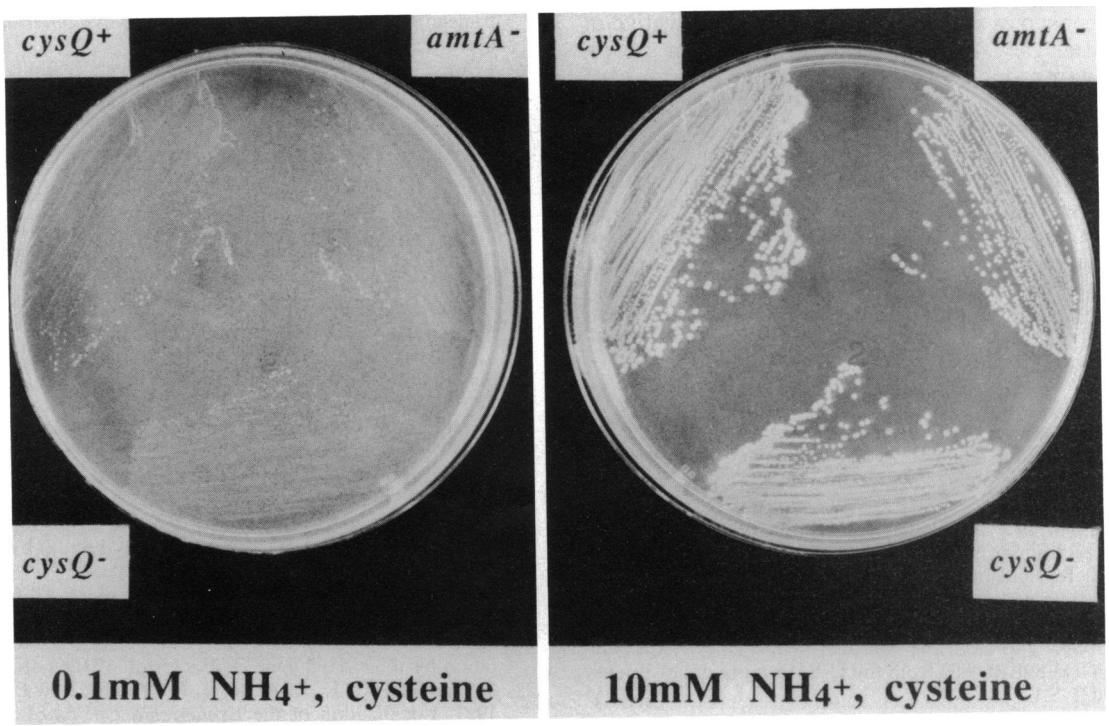

FIG. 6. Comparison of wild-type and *cysQ* and *amtA* mutant bacterial strains on modified M9 minimal medium and either low (0.1 mM) or normal (10 mM) concentrations of ammonium acetate. Strains: *cysQ*$^+$, ET8000; *cysQ* mutant, ET8000 *cysQ*::*kan* (DB6908); *amtA* mutant, ET8000 *amtA*::Tn*10* (AJ2653) grown in medium containing 0.2 mM cysteine. The plate with 10 mM ammonium was incubated for 1 day and the plate with 0.1 mM ammonium was incubated for 2 days at 37°C before being photographed. Identical growth patterns were obtained with sulfite in place of cysteine, but only Cys$^+$ parental strains grew on minimal low-ammonium medium lacking sulfite or cysteine. Equivalent weak growth of mutant and Cys$^+$ sibling strains on the low-ammonium medium was also observed with all other lineages tested (DB747 and its *cysQ* derivative DB5656, MG1655 and its *cysQ* derivative DB6316, TG1 and its *cysQ* derivative DB6913). Growth was weaker on medium containing 0.025 mM rather than 0.1 mM ammonium acetate, as expected, since ammonium was limiting. In the cases of Cys$^+$ strains, this slow growth was not stimulated by adding 10 or 20 mM cysteine (or sulfite or thiosulfate), whereas growth was stimulated by adding 20 mM glutamate or arginine (which serve as ammonium sources [53]).

the medium) and for cysteine (because of a mutation). Although the inefficient induced methylammonium uptake by *amtA* cells was also interpreted to reflect a specific uptake defect, the reported data (21) indicate that the basal level of uptake was not affected by the *amtA* mutation. Earlier work had shown that induction by growth in arginine reflects the slow release of ammonium from this source, relative to the rate of ammonium consumption (45, 53). Because the poor growth of *cysQ* (*amtA*) mutants on cysteine-free medium should allow the arginine-derived ammonium to accumulate to repressing levels, we do not find it necessary to postulate a role for CysQ (AmtA) in ammonium or methylammonium uptake.

How does CysQ act in the synthesis of sulfite and cysteine? Several possible roles have been eliminated by our results to date: (i) sulfate uptake, (ii) stabilization of ATP sulfurylase, (iii) synthesis or stabilization of the ATP sulfurylase activator, and (iv) modulation of CpdB. In addition, the *cysQ* sequence does not match that of *ppa*, the gene for pyrophosphatase (30), an enzyme probably needed for efficient APS synthesis (Fig. 1). The leakiness of *cysQ*-null alleles in many strain backgrounds might reflect (i) a second gene with a functionally related role; (ii) an intrinsic activity of gene(s) that can mutate to give a Cys$^+$ revertant phenotype; or, if *cysQ* is regulatory, (iii) strain background-dependent differences in the quantitative effects of CysQ on the gene, protein, or metabolite that is the target of its control.

Studies of Cys$^+$ revertants are providing insights into how CysQ may act. Several spontaneous reversion mutations were mapped to a region that includes *cysTWA* permease genes, were recessive to the wild-type (nonrevertant) alleles, and were defective in sulfate uptake. These results suggested that reversion results from loss of function, not from an unusual expression of a silent or cryptic suppressor gene. Accordingly, we have begun to isolate transposon insertions that restore prototrophy to *cysQ* mutants in a nonleaky background (50). One insertion that resulted in very small colonies on cysteine-free medium was in *cysA*, which encodes a subunit of sulfate permease (49). Transduction of this insertion into a *cysQ*$^+$ strain resulted in the same small-colony phenotype, indicating that the phenotype reflected loss of *cysA* function, not poor suppression of the *cysQ* mutation. A second insertion, which resulted in colonies of nearly normal size, was in *cysP*, a gene whose product contributes to efficient sulfate and thiosulfate binding (19). In interpreting these reversion data we draw on early findings that mutations in *cysH* or in *trxA* plus *grx* cause poor growth, apparently because accumulated PAPS or a derivative of it is toxic, and that these mutations can be compensated by mutations inactivating sulfate permease (16, 45a). Although the role of *cysP* in the *cys* pathway is not understood, the ability of permease mutations to compensate for the defect in *cysQ* suggests that CysQ also acts on PAPS. Perhaps CysQ participates with the CysH sulfotransferase to generate sulfite. Alternatively, perhaps CysQ se-

questers or consumes excess PAPS or a toxic derivative of it. On this latter view, cysteine might be needed for growth of *cysQ* mutants only to allow repression of *cys* gene expression and thereby decrease PAPS synthesis, rather than to compensate for a missing biosynthetic enzyme. CysQ exhibits striking amino acid sequence homology to mammalian inositol monophosphatase as well as to the product of the *suhB* gene of *E. coli* (Fig. 6) (39). The homology between CysQ and inositol monophosphatase, in particular, encourages models in which CysQ acts on a phosphorylated metabolite such as PAPS, possibly ensuring that it plays its essential biosynthetic role without toxicity to the cell.

ACKNOWLEDGMENTS

We thank C. M. Berg, H. V. Huang, S. H. Phadnis, M. Russel, and P. M. Wikström for stimulating discussions and A. Jayakumar and E. Barnes for the exchange of strains and unpublished information.

A.F.N. was a trainee in Medicine (Infectious Diseases) (Public Health Service) training grant 5 T32 AI07172 from the National Institute of Allergy and Infectious Diseases) and in Medical Informatics (NLM training grant 5-TI-LM07049). This work was supported by Public Health Service grants GM-37138, HG00563, BRSG 613-4743, and DK43003 from the National Institutes of Health and by grant DEFGO2-89ER60862 from the U.S. Department of Energy to D.E.B. and T.S.L.

REFERENCES

1. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54**:130–197.
2. **Bairoch, A.** 1991. PROSITE: a dictionary of sites and patterns in proteins. Nucleic Acids Res. **19**:2241–2245.
3. **Barret, E. L., and G. W. Chang.** 1979. Cysteine auxotrophs of *Salmonella typhimurium* which grow without cysteine in a hydrogen/carbon dioxide atmosphere. J. Gen. Microbiol. **115**: 513–516.
4. **Beacham, I. R., and S. Garrett.** 1980. Isolation of *Escherichia coli* mutants (*cpdB*) deficient in periplasmic 2′:3′-cyclic phosphodiesterase and genetic mapping of the *cpdB* locus. J. Gen. Microbiol. **119**:31–34.
5. **Berg, D. E., A. Weiss, and L. Crossland.** 1980. Polarity of Tn*5* insertion mutations in *Escherichia coli*. J. Bacteriol. **142**:439–446.
6. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, and H. W. Boyer.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
7. **Campbell, A.** 1961. Sensitive mutants of bacteriophage λ. Virology **14**:22–32.
8. **Casadaban, M. J., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J. Mol. Biol. **138**:179–207.
9. **Chow, W.-Y., and D. E. Berg.** 1988. Tn*5tacI*, a derivative of transposon Tn*5* that generates conditional mutations. Proc. Natl. Acad. Sci. USA **85**:6468–6472.
10. **Close, T. J., and R. L. Rodriguez.** 1982. Construction and characterization of the chloramphenicol-resistance gene cartridge: a new approach to the transcriptional mapping of extrachromosomal elements. Gene **20**:305–316.
11. **Csonka, L. N., and A. J. Clark.** 1979. Deletions generated by the transposon Tn*10* in the *srl recA* region of the *Escherichia coli* K-12 chromosome. Genetics **93**:321–343.
12. **Davis, R. W., D. Botstein, and J. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Diehl, R. E., P. Whiting, J. Potter, N. Gee, C. I. Ragan, D. Linemeyer, R. Schoepfer, C. Bennett, and R. A. F. Dixon.** 1990. Cloning and expression of bovine brain inositol monophosphatase. J. Biol. Chem. **265**:5946–5949.
14. **Dreyfuss, J.** 1964. Characterization of a sulfate and thiosulfate-transporting system from *Salmonella typhimurium*. J. Biol. Chem. **239**:2292–2297.
15. **Fabiny, J. M., A. Jayakumar, A. C. Chinault, and E. M. Barnes.** 1991. Ammonium transport in *Escherichia coli*: localization and nucleotide sequence of the *amtA* gene. J. Gen. Microbiol. **137**:983–989.
16. **Gillespie, D., M. Demerec, and H. Itakawa.** 1968. Appearance of double mutants in aged cultures of *Salmonella typhimurium* cysteine-requiring strains. Genetics **59**:433–442.
17. **Guyer, M. S., R. R. Reed, J. A. Steitz, and K. B. Low.** 1981. Identification of a sex-factor-affinity site in *E. coli* as γδ. Cold Spring Harbor Symp. Quant. Biol. **45**:135–139.
18. **Hrniewicz, M., and N. M. Kredich.** 1991. The *cysP* promoter of *Salmonella typhimurium*: characterization of two binding sites for CysB protein, studies of in vivo transcription initiation, and demonstration of anti-inducer effects of thiosulfate. J. Bacteriol. **173**:5876–5886.
19. **Hrniewicz, M., A. Sirko, A. Palucha, A. Bock, and D. Hulanicka.** 1990. Sulfate and thiosulfate transport in *Escherichia coli* K-12: identification of a gene encoding a novel protein involved in thiosulfate binding. J. Bacteriol. **172**:3358–3366.
20. **Huang, C. J., and E. L. Barrett.** 1990. Identification and cloning of genes involved in anaerobic sulfite reduction by *Salmonella typhimurium*. J. Bacteriol. **172**:4100–4102.
21. **Jayakumar, A., S. J. Hwang, J. M. Fabiny, A. C. Chinault, and E. M. Barnes.** 1989. Isolation of an ammonium or methylammonium ion transport mutant of *Escherichia coli* and complementation by the cloned gene. J. Bacteriol. **171**:996–1001.
22. **Jayakumar, A., K. E. Rudd, J. M. Fabiny, and E. M. Barnes.** 1991. Localization of the *Escherichia coli amtA* gene to 95.8 minutes. J. Bacteriol. **173**:1572–1573.
23. **Kohara, Y.** 1990. Correlation between the physical and genetic maps of the *Escherichia coli* K-12 chromosome, p. 29–42. *In* K. Drlica and M. Riley (ed.), The bacterial chromosome. American Society for Microbiology, Washington, D.C.
24. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50**:495–508.
25. **Kredich, N. M.** 1987. Biosynthesis of cysteine, p. 419–428. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
26. **Kredich, N. M., L. J. Foote, and M. D. Hulanicka.** 1975. Studies on the mechanism of inhibition of *Salmonella typhimurium* by 1,2,4-triazole. J. Biol. Chem. **250**:7324–7331.
27. **Krishnan, B. R., D. Kersulyte, I. Brikun, H. V. Huang, C. M. Berg, and D. E. Berg.** 1992. Transposon- and PCR-based sequencing of DNAs cloned in λ phage. Methods Enzymol., in press.
28. **Kulakauskas, S., P. M. Wikström, and D. E. Berg.** 1991. Efficient introduction of cloned mutant alleles into the *Escherichia coli* chromosome. J. Bacteriol. **173**:2633–2638.
29. **Kurnit, D. M., and B. Seed.** 1990. Improved genetic selection for screening bacteriophage libraries by homologous recombination in vivo. Proc. Natl. Acad. Sci. USA **87**:3166–3169.
30. **Lahti, R., T. Pitkäranta, E. Valve, I. Ilta, E. Kukko-Kalske, and J. Heinonen.** 1988. Cloning and characterization of the gene encoding inorganic pyrophosphatase of *Escherichia coli* K-12. J. Bacteriol. **170**:5901–5907.
31. **Leyh, T. S.** 1990. The discovery of a coenzyme that stimulates the activity of ATP sulfurylase from *E. coli* K-12. FASEB J. **4**:A1988 (abstr. 1717).
32. **Leyh, T. S., and G. D. Markham.** Unpublished data.
33. **Leyh, T. S., and Y. Suo.** 1992. GTPase mediated activation of ATP sulfurylase. J. Biol. Chem., in press.
34. **Leyh, T. S., J. C. Taylor, and G. D. Markham.** 1988. The sulfate activation locus of *Escherichia coli* K-12: cloning, genetic, and enzymatic characterization. J. Biol. Chem. **263**:2409–2416.
35. **Liu, J., and I. R. Beacham.** 1990. Transcription and regulation of the *cpdB* gene in *Escherichia coli* K12 and *Salmonella typhmurium* LT2: evidence for modulation of constitutive promoters by

cyclic AMP-CRP complex. Mol. Gen. Genet. **222**:161–165.
36. **Liu, J., D. M. Burns, and I. R. Beacham.** 1986. Isolation and sequence analysis of the gene (*cpdB*) encoding periplasmic 2′,3′-cyclic phosphodiesterase. J. Bacteriol. **165**:1002–1010.
37. **MacNeil, T., D. MacNeil, and B. Tyler.** 1982. Fine-structure map and complementation analysis of the *glnA-glnL-glnG* region in *Escherichia coli*. J. Bacteriol. **150**:1302–1313.
38. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–78.
39. **Neuwald, A. F., J. D. York, and P. W. Majerus.** 1991. Diverse proteins homologous to inositol monophosphatase. FEBS Lett. **294**:16–18.
40. **Pardee, A. B., L. S. Prestidge, M. B. Whipple, and J. Dreyfuss.** 1966. A binding site for sulfate and its relation sulfate transport into *Salmonella typhimurium*. J. Biol. Chem. **241**:3962–3969.
41. **Parra, F., P. Britton, C. Castle, M. C. Jones-Mortimer, and H. L. Kornberg.** 1983. Two separate genes involved in sulphate transport in *Escherichia coli* K12. J. Gen. Microbiol. **129**:357–358.
42. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85**:2444–2448.
43. **Phadnis, S. H., H. V. Huang, and D. E. Berg.** 1989. Tn*5supF*, a 264-base-pair transposon derived from Tn*5* for insertion mutagenesis and sequencing DNAs cloned in phage λ. Proc. Natl. Acad. Sci. USA **86**:5908–5912.
44. **Phadnis, S. H., S. Kulakauskas, B. R. Krishnan, J. Hiemstra, and D. E. Berg.** 1991. Transposon Tn*5supF*-based reverse genetic method for mutational analysis of *Escherichia coli* with DNAs cloned in λ phage. J. Bacteriol. **173**:896–899.
45. **Reitzer, L. J., and B. Magasanik.** 1989. Ammonia assimilation and the biosynthesis of glutamine, glutamate, aspartate, asparagine, L-alanine, and D-alanine, p. 302–320. *In* F. C. Neidhardt J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
45a. **Russel, M., P. Model, and A. Holmgren.** 1990. Thioredoxin or glutaredoxin in *Escherichia coli* is essential for sulfate reduction, but not for deoxyribonucleotide synthesis. J. Bacteriol. **172**:1923–1929.
46. **Satishchandran, C., and G. D. Markham.** 1989. Adenosine-5′-phosphosulfate kinase from *Escherichia coli* K-12. J. Biol. Chem. **264**:15012–15021.
47. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
48. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53**:1–24.
49. **Sirko, A., M. Hrniewicz, D. Hulanicka, and A. Bock.** 1990. Sulfate and thiosulfate transport in *Escherichia coli* K-12: nucleotide sequence and expression of the *cysTWAM* gene cluster. J. Bacteriol. **172**:3351–3357.
50. **Suziedelis, K., S. Kulakauskas, and D. E. Berg.** Unpublished data.
51. **Tsang, M. L.-S., and J. A. Schiff.** 1976. Sulfate-reducing pathway in *Escherichia coli* involving bound intermediates. J. Bacteriol. **125**:923–933.
52. **Tsang, M. L.-S.** 1981. Assimilatory sulfate reduction in *Escherichia coli*: identification of the alternate cofactor for adenosine 3′-phosphate 5′-phosphosulfate reductase as glutaredoxin. J. Bacteriol. **146**:1059–1066.
53. **Tyler, B.** 1978. Regulation of the assimilation of nitrogen compounds. Annu. Rev. Biochem. **47**:1127–1162.
54. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
55. **Wanner, B. L.** 1986. Novel regulatory mutants of the phosphate regulon of *Escherichia coli* K-12. J. Mol. Biol. **191**:39–58.
56. **Yano, R., H. Nagain, K. Shiba, and T. Yura.** 1990. A mutation that enhances synthesis of σ^{32} and suppresses temperature-sensitive growth of the *rpoH15* mutant of *Escherichia coli*. J. Bacteriol. **172**:2124–2130.